March 25, 1996, (abandoned); U.S. patent application Serial No. 08/621,859, filed March 25, 1996, now U.S. Patent No. 6,117,679; U.S. patent application Serial No. 08/537,874, filed March 4, 1996, now U.S. Patent No. 5,830,721 (U.S. National Phase of PCT/US95/02126, filed February 17, 1995); and U.S. patent application Serial No. 08/198,431, filed February 17, 1994, now U.S. Patent No. 5,605,793.

All other priority claims are **withdrawn** without prejudice to subsequent renewal

CONCERNING THE STATUS OF THE CLAIMS.

Claims 31-82, 84-104, 106-148, and 150-169 are pending with entry of this amendment. Claim 83 is cancelled, claims 31 and 33 are amended, and claims 153-169 are added herein. These amendments introduce no new matter and support is replete throughout the specification.

Claim 83 has been cancelled because it is an inadvertent duplicate of claim 52.

With respect to claims 31 and 33, support for "homologous recombination sites" can be found throughout the specification. For example, see the specification at page 11, line 23 through page 12, line 14. With respect to new claim 161, support for "multi-enzyme pathway" is found at, for example, page 11, lines 19-21.

New dependent claims 153-160 and 162-169 are analogous to previously pending dependent claims.

Applicants submit that no new matter has been added to the application by way of the above Amendment. Accordingly, entry of the Amendment is respectfully requested.

CONCERNING THE INFORMATION DISCLOSURE STATEMENT.

Applicants note with appreciation the Examiner's thorough consideration of the references cited in the Information Disclosure Statement (Form 1449) submitted on January 24, 2000. Applicants note that this is the second consideration of this IDS, which was also attached to the August 1, 2000 Office Action. **Applicants note that the IDS submitted on December 19, 2000 has not yet been indicated to be considered.**

CONCERNING 35 U.S.C. §102(E).

Claims 31-104, 106-148, and 150-152 were rejected under 35 U.S.C. §102(e) as allegedly anticipated by Jerrell (US Patent No. 5,498,531 (1994).

Applicants submit that the amendments to the claims overcome these rejections. In order for a reference to anticipate an invention, the reference must teach each and every element of the claimed invention. Jerrell does not teach each and every element of amended claims 31 and 33. Jerrell teaches manipulating nucleic acids by using intron sequences to mediate specific cleavage and ligation of discontinuous nucleic acids. For example, see Jerrell, column 29, lines 56 through column 30, line 19 for generation of a plasmid-nucleic acid construct by including intron II sequences at the ends of both the cut plasmid and the nucleic acid and subjecting them to a transplicing reaction. Nowhere does Jerrell teach a method for recombination that uses homologous recombination sequences such as that recited in amended claims 31 and 33. Accordingly, Jerrell does not anticipate independent claims 31 and 33, nor dependent claims 32, 34-104, 106-148, and 150-152. Applicants respectfully request withdrawal of this rejection of the claims.

CONCERNING 35 U.S.C. §102/§103(A).

Claims 31-104, 106-148, and 150-152 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by or in the alternative under 35 U.S.C. §103(a) as allegedly obvious over Stemmer (Nature, 37:389-391 (1994)). In addition, claims 31-104, 106-148, and 150-152 were rejected under 35 U.S.C. §102(a) as allegedly anticipated by or in the alternative under 35 U.S.C. §103(a) as allegedly obvious over Cremari et al., (Nature Biotechnology, 14:315-319 (1996)). For both pieces of art, the Examiner stated that the art "...does not explicitly use the phrase "conjoining a plurality of recombination sites to a plurality of subsequences of at least one nucleic acid to thereby produce a plurality of recombination cassettes" to describe his invention. However this is exactly what is occurring during the early steps/cycle of the method described . . .Therefore absent a showing to the contrary, this limitation is considered to be inherent to the method described . . .." (July 5, 2001 Office Action , points 6 and 7.)

Applicants respectfully traverse. The limitation of "conjoining a plurality of recombination sites to a plurality of subsequences of at least one nucleic acid to thereby produce a plurality of recombination cassettes" is not described in either Stemmer or Cremari. Stemmer and Cremari describe a recombination method that utilizes sequence homology found in the nucleic acid sequences being recombined. In contrast, the claimed method provides sequence homology between the nucleic acids to be recombined by adding "homologous recombination sites," e.g., beginning at page 11, line 19:

> Gene clusters such as those involved in polyketide synthesis (or indeed any multi-enzyme pathways catalyzing analogous metabolic reactions) can be recombined by recursive sequence recombination *even if they lack DNA sequence homology. Homology can be introduced using synthetic oligonucleotides as PCR primers*. In addition to the specific sequences for the gene being amplified, all of the primers used to amplify one type of enzyme (for example the acyl carrier protein in polyketide synthesis) are synthesized to contain an additional sequence of 20-40 bases 5' to the gene (sequence A) and a different 20-40 base sequence 3' to the gene (sequence B). The adjacent gene (in this case the keto-synthase) is amplified using a 5' primer which contains the complementary strand of sequence B (sequence B'), and a 3' primer containing a different 20-40 base sequence (C). Similarly, primers for the next adjacent gene (keto-reductases) contain sequences C' (complementary to C) and D. If 5 different polyketide gene clusters are being shuffled, all five acyl carrier proteins are flanked by sequences A and B following their PCR amplification. *In this way, small regions of homology are introduced, making the gene clusters into site-specific recombination cassettes*. Subsequent to the initial amplification of individual genes, the amplified genes can then be mixed and subjected to primerless PCR. Sequence B at the 3' end of all of the five acyl carrier protein genes can anneal with and prime DNA synthesis from sequence B' at the 5' end of all five keto reductase genes. In this way all possible combinations of genes within the cluster can be obtained. *Oligonucleotides allow such recombinants to be obtained in the absence of sufficient sequence homology for recursive sequence recombination described above*. Only homology of function is required to produce functional gene clusters. (emphasis added)

In contrast to the above teaching, neither reference teaches the addition of small regions of homology to the nucleic acids being recombined. Accordingly, neither Stemmer nor Cremari teach each and every element of the claimed invention, and Applicants respectfully request withdrawal of this rejection.

CONCERNING THE NON-STATUTORY DOUBLE PATENTING REJECTION

The claims were rejected for alleged non-statutory obviousness-type double patenting. Applicants note that a Terminal Disclaimer will be submitted, if appropriate, when the claims are indicated to otherwise be in condition for allowance.

CONCLUSION

In view of the foregoing, Applicants believes all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 337-7871.

LAW OFFICES OF JONATHAN ALAN QUINE
P.O. Box 458
Alameda, CA 94501
Tel: 510 337-7871
Fax: 510 337-7877

Respectfully submitted,

Susan T. Hubl
Reg. No: 47,668

APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE ILLUSTRATING THE AMENDMENTS MADE TO THE SPECIFICATION AND CLAIMS OF 09/490,643

IN THE SPECIFICATION

The first paragraph has been amended as follows:

This application is a continuation of U.S. patent application Serial No. 09/189,103, filed November 9, 1998, which is a continuation of U.S. patent application Serial No. 08/650,400, filed May 20, 1996, now U.S. Patent No. 5,837,458, which is a continuation-in-part of U.S. patent application Serial No. 08/621,430, filed March 25, 1996, (abandoned), and of U.S. patent application Serial No. 08/621,859, filed March 25, 1996, now U.S. Patent No. 6,117,679, and of U.S. patent application Serial No. 08/537,874, filed March 4, 1996, now U.S. Patent No. 5,830,721 (U.S. National Phase of PCT/US95/02126, filed February 17, 1995), and of U.S. patent application Serial No. 08/198,431, filed February 17, 1994, now U.S. Patent No. 5,605,793, ~~which is a continuation-in-part of U.S. patent application Serial No. 08/198,431, filed February 17, 1994, Serial No. PCT/US95/02126, filed February 17, 1995, Serial No. 08/537,874, filed October 30, 1995, Serial No. 08/621,859, filed March 25, 1996, Serial No. 08/621,430, filed March 25, 1996, and Serial No. 08/425,684~~ the specifications of which are incorporated herein by reference in their entirety for all purposes.

IN THE CLAIMS

31. (Amended) A method for combinatorial cassette-based recombination, comprising:
conjoining a plurality of homologous recombination sites to a plurality of subsequences of at least one nucleic acid, thereby producing a plurality of recombination cassettes;
recombining the recombination cassettes, or fragments thereof, at the recombination sites, thereby producing a plurality of permutations of the recombination cassettes within a plurality of resulting recombinant nucleic acids; and,
selecting the plurality of recombinant nucleic acids for one or more property or encoded activity.

33. (Amended) A method for permuting subsequences of interest in at least one nucleic acid, the method comprising:

identifying functionally similar subsequences in the at least one nucleic acid;
conjoining at least one homologous recombination site to a plurality of the functionally similar subsequences, thereby producing a plurality of recombination cassettes; and,
recombining the recombination cassettes, or fragments thereof, at the recombination sites, thereby producing a plurality of permutations of the recombination cassettes within a plurality of resulting recombinant nucleic acids.

83. (Cancelled) ~~The method of claim 31 or 33, wherein the at least one nucleic acid encodes a multi-subunit enzyme.~~

153. (New) The method of claim 51, further comprising recombining one or more of the plurality of recombinant nucleic acids with one or more additional nucleic acid.

154. (New) The method of claim 51, further comprising fragmenting the recombination cassettes with a nuclease prior to said recombining step, wherein said recombining step is performed by primerless PCR.

155. (New) The method of claim 51, wherein the at least one nucleic acid comprises one or more sequence produced by in vitro sequence recombination.

156. (New) The method of claim 51, wherein the at least one nucleic acid comprises one or more sequences produced by recursive in vitro recombination.

157. (New) The method of claim 51, wherein the at least one nucleic acid is produced by in vivo recombination.

158. (New) The method of claim 51, wherein the at least one nucleic acid is produced by recursive in vivo sequence recombination.

159. (New) The method of claim 51, wherein the plurality of recombination cassettes comprise subsequences which are allelic or species variants.

160. (New) The method of claim 51, wherein the at least one nucleic acid is selected from one or more libraries of nucleic acids derived from one or more of: a bacteria, an Alcaligenes, a Zoogloea, a Rhizobium, a Bacillus, an Azobacter, or a eukaryote.

161. (New) The method of claim 51 wherein, the gene cluster encodes a multi-enzyme pathway.

162. (New) The method of claim 161, further comprising recombining one or more of the plurality of recombinant nucleic acids with one or more additional nucleic acid.

163. (New) The method of claim 161, further comprising fragmenting the recombination cassettes with a nuclease prior to said recombining step, wherein said recombining step is performed by primerless PCR.

164. (New) The method of claim 161, wherein the at least one nucleic acid comprises one or more sequence produced by in vitro sequence recombination.

165. (New) The method of claim 161, wherein the at least one nucleic acid comprises one or more sequences produced by recursive in vitro recombination.

166. (New) The method of claim 161, wherein the at least one nucleic acid is produced by in vivo recombination.

167. (New) The method of claim 161, wherein the at least one nucleic acid is produced by recursive in vivo sequence recombination.

168. (New) The method of claim 161, wherein the plurality of recombination cassettes comprise subsequences which are allelic or species variants.

169. (New) The method of claim 161, wherein the at least one nucleic acid is selected from one or more libraries of nucleic acids derived from one or more of: a bacteria, an Alcaligenes, a Zoogloea, a Rhizobium, a Bacillus, an Azobacter, or a eukaryote.